

GRANT NUMBER DAMD17-94-J-4177

TITLE: Breast Cancer Resource for Research and Banking, with Emphasis on Early Tumors and Precursor Lesions

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REPORT DATE: October 1998

TYPE OF REPORT: Final

PREPARED FOR: Commander  
U.S. Army Medical Research and Materiel Command  
Fort Detrick, Frederick, Maryland 21702-5012

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19990401 054

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE October 1998	3. REPORT TYPE AND DATES COVERED Final (1 Dec 94 - 1 Sep 98)	
4. TITLE AND SUBTITLE Breast Cancer Resource for Research and Banking, with Emphasis on Early Tumors and Precursor Lesions			5. FUNDING NUMBERS DAMD17-94-J-4177	
6. AUTHOR(S)  Helen Feiner, M.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  New York University Medical Center New York, New York 10016			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Commander U.S. Army Medical Research and Materiel Command Fort Detrick, Frederick, Maryland 21702-5012			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT  Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200)  The Breast Cancer Resource for Research and Banking has accrued breast cells or tissues from 1,467 patients during the grant period (3 year grant with 9 month extension without additional funding). The emphasis of this project has been on the collection of microscopic at risk and precursor lesions as imprints/scrapes. Additionally, throughout the grant period, all invasive carcinomas with available tissue have been accrued, since most investigators who have requested samples have requested frozen pieces of tumor tissue paired with normal tissue from the same patient, i.e. their interest has been in established carcinomas and not in precursor lesions. During the last year we also filled requests for specimens for microdissection. Over the entire grant period 26 investigator requests for tissue have been filled. At termination of the grant the Breast Cancer Resource was transferred to the auspices of the Resource for Tumor Tissue and Data of the NYU Kaplan Comprehensive Cancer Center.				
14. SUBJECT TERMS  Breast Cancer			15. NUMBER OF PAGES 34	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

## FOREWORD

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John F. Fawcett MD 9/28/98  
PI - Signature Date

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## INTRODUCTION

Basic, clinical, and translational research on breast cancer in the United States has been stimulated in recent years by increased government and private funding. Translational research, in particular, requires the availability of human breast cancer tissue, as well as breast tissue with "precursor" and "at risk" lesions. At risk lesions are the proliferative and atypical proliferative components of fibrocystic change, and the precursor lesion is carcinoma in situ. These lesions have been defined histologically and their roles in breast carcinogenesis have been validated epidemiologically. This grant was funded in the category of "Infrastructure Enhancement" specifically to make breast cancer tissue, precursor, and at risk lesions available to investigators in the field of human breast carcinogenesis.

## METHODS

Clinical cancers, that is invasive carcinomas that have resulted in a palpable mass lesion, were banked in standard fashion as snap-frozen pieces of tissue, together with pieces of non-neoplastic breast tissue from the same patient, and a portion of lymph node when available.

Collecting at risk and precursor lesions of breast cancer, which are almost invariably microscopic, is difficult, firstly because the lesions are so small, and secondly because good medical practice requires that all the tissue excised be subjected to routine histopathologic examination in order to properly classify the lesion.

Accordingly, in years 1 to 3 of the grant we collected at risk and precursor lesions of breast cancer as slide imprints/scrapes prepared from excised breast tissue prior to histopathologic examination. By the end of year two 782 imprint samples had been collected from mammographically detected (non-palpable) lesions. These covered the spectrum of fibrocystic change (non-proliferative, proliferative, and proliferative with atypia), as well as ductal carcinoma in situ and lobular carcinoma in situ. It was disappointing that despite considerable effort to publicize this collection of material, investigators did not request it. The reasons were twofold. Firstly, most basic scientists are unfamiliar with the histopathologically defined at risk and precursor lesions of breast cancer; they are more interested in established cancers (mass lesions). Secondly, the samples are small and comprised of mixtures of cells (of necessity stromal cells and lymphohistiocytic cells are admixed with lesional cells in imprint/aspirate specimens). At about the same time the technique of microdissection was evolving, and provided an alternate method for acquiring such lesions for research purposes. Accordingly in year 3 and in the extension period we have been supplying investigators with material prepared for microdissection. This technique allows one to obtain pure specimens of microscopic precursor and at risk lesions, from either fixed paraffin embedded tissue (for DNA - PCR studies) or from frozen sections (for RNA based studies). Specimens obtained by microdissection are superior to imprints and aspirates inasmuch as the histologic context from which samples are obtained can be documented, and the samples are pure. Because of the aforesaid, in year 3 we turned our efforts away from imprints and toward providing samples for microdissection.

Use of the Resource has been stimulated by the award of 16 pilot projects from developmental funds from the Kaplan Comprehensive Cancer Center's NCI Breast Cancer Program Grant during the 1995-1998 period.

Outside NYU, the Resource has been included in the Breast Cancer Specimen and Data Information System, a collaborative project sponsored by the National Action Plan for Breast Cancer Biologic Resources Banks Working Group and the NCI. The DOD Breast Cancer Research Program "Era of Hope" in Washington D.C. in October/November 1997 provided another forum for publicizing the Resource.

To obtain feedback on the satisfaction of investigators with the material sent to them, two contacts are made with each recipient. The first is to determine the state of material given or shipped and occurs within a day or two of shipping. The second contact is made 6 - 18 months later to determine the level of satisfaction in terms of results obtained. User files are maintained for each recipient of samples. User records are initiated with "Investigator Request" forms (Appendix 1).

To obtain information on the quality and durability of banked tissues and cells, specimens obtained in 1995, 1996 and 1997 have been subjected to a variety of analyses. These analyses were immunohistochemistry, immunofluorescence microscopy, fluorescence in situ hybridization, and RT-PCR. Analyses were done in various laboratories at NYU that have expertise in these assays. Records are maintained on "Evaluation of Banked Material" forms (Appendix 2).

The Resource technician has also culled the NYU departmental records retrospectively so that all patients with mammographically detected lesions from 1991-1994 have been entered into the database. Even though no fresh samples (imprints/aspirates) are available in these cases, the ability to microdissect the archival samples has made them a valuable addition to the Resource.

## RESULTS

The numbers of the various types of breast tissue samples that have been banked and entered into our database during each grant year, as well as the cumulative numbers of samples for the entire collection period are shown in Tables 1 to 4. Tables 1 and 2 use the format of previous annual reports. Tables 3 and 4 show data for all four years.

In Table 1 the breakdown is by type of samples available. In Table 2 the breakdown is by type of lesion as defined histopathologically. Total number of samples in Table 1 exceeds total number of cases in Table 2 because some cases (patients) generated more than one sample type.

TABLE 1

BANKED CASES OF BREAST CANCER AND PRECURSOR LESIONS  
AT NYU MEDICAL CENTER BY SAMPLE TYPES

	<u>Grant Year #4</u> <u>12/97 - 8/98</u>	<u>4 Yr. Cumulative</u> <u>12/94 - 8/98</u>
Imprints/scrapes	0	633
Aspirated cells	115	642
Snap frozen tissue fragments*	<u>69</u>	<u>757</u>
TOTAL	184	2,032

\*includes 308 paired samples of breast cancers with normal tissue.

TABLE 2

BANKED CASES OF BREAST CANCER AND PRECURSOR LESIONS  
AT NYU MEDICAL CENTER BY HISTOPATHOLOGIC DIAGNOSIS

	<u>Grant Year #4</u> <u>12/97 - 8/98</u>	<u>4 Yr. Cumulative</u> <u>12/94 - 8/98</u>
Invasive ductal carcinoma	67	383
Invasive lobular carcinoma	12	59
Ductal carcinoma in situ*	7	151
Lobular carcinoma in situ*	0	46
Secondary carcinoma, lymph node	27	110
Lymph node without tumor	0	81
Fibrocystic change, non-proliferative	1	190
Fibrocystic change, proliferative**	1	215
Fibrocystic change, proliferative with atypia**	0	80
Other (mostly fibroadenoma)	<u>13</u>	<u>153</u>
TOTAL	128	1,468

\*precursor lesion      \*\*at risk lesion

Table 5 indicates the number of patients from whom samples were obtained during years 1 and 3 and the extension period, and during the entire grant period. Table 6 indicates the numbers of requests for specimens that have been filled over similar time periods.

TABLE 3

SPECIMENS BY SAMPLE TYPE

	<u>1995</u>	<u>1996</u>	<u>1997</u>	<u>1998</u>	<u>Total</u>
Imprints/TP	367	415	102	0	633
Aspirates	149	219	159	115	642
Tissue	<u>233</u>	<u>199</u>	<u>256</u>	<u>69</u>	<u>757</u>
TOTAL	749	833	517	184	2,032

TABLE 4

SPECIMENS BY HISTOPATHOLOGIC DIAGNOSIS

	<u>1995</u>	<u>1996</u>	<u>1997</u>	<u>1998</u>	<u>Total</u>
Invasive ductal carcinoma	118	112	86	67	383
Invasive lobular carcinoma	16	20	11	12	59
In situ ductal	55	50	39	7	151
In situ lobular	11	25	10	0	46
Secondary carcinoma	25	23	35	27	110
FCD - proliferative	86	92	90	1	269
FCD - non-proliferative	71	107	11	1	190
Other	<u>48</u>	<u>104</u>	<u>97</u>	<u>13</u>	<u>262</u>
TOTAL	430	533	379	128	1,470

TABLE 5

NUMBER OF PATIENTS WITH BANKED SAMPLES

<u>Grant Year #1</u>	<u>Grant Year #2</u>	<u>Grant Year #3</u>	<u>Grant Year #4</u>	<u>4 Yr. Cumulative</u>
<u>12/94 - 11/95</u>	<u>12/95 - 11/96</u>	<u>12/96 - 11/97</u>	<u>12/97 - 8/98</u>	<u>12/94 - 8/98</u>
430	537	365	135	1,467

TABLE 6

REQUESTS FOR SPECIMENS FILLED

	Grant Yr. #1	Grant Yr. #2	Grant Yr. #3	Grant Yr. #4	4 Yr. Cumulative
	<u>12/94 - 11/95</u>	<u>12/95 - 11/96</u>	<u>12/96 - 11/97</u>	<u>12/97 - 8/98</u>	<u>12/94 - 8/98</u>
Imprints/scrapes	1	1	0	0	2
Frozen tissue	2	4	9	4	19
Tissue for microdissection	<u>0</u>	<u>0</u>	<u>2</u>	<u>3</u>	<u>5</u>
TOTAL	3	5	11	7	26

As shown in Table 3, we reduced the numbers of imprint/scrape samples collected in years 3 and 4 of the grant. These represent samples of microscopic lesions, mainly in situ carcinoma and proliferative fibrocystic changes. The reason for this reduced collection is twofold. Firstly, we now have a large collection of these lesions and requests for such samples have been very low. Secondly, the technique of microdissection has been gaining increasing favor as an alternative method for obtaining samples of microscopic lesions. Current amplification techniques allow the analysis of cells from a single microdissected duct or lobule of breast tissue. Microdissection can be done on frozen or on fixed, paraffin embedded tissue. Furthermore, the purity of specimens can be monitored by examination of sections before and after the microdissection is done. The success of this technique may be the reason for the underutilization of our imprint/scrape samples. Prior to the use of microdissection, scrapes/imprints represented the only means for obtaining precancerous and microscopic breast lesions for research purposes. The disadvantages of imprint/scrapes as compared to microdissection relates to the fact that imprint/scrape samples represent mixtures of cells, albeit the lesional cells predominate. In both instances the samples are small, but investigators prefer to use samples of known and verifiable purity.

There have been several opportunities for publicizing the Resource at NYU. It has been written up three times in the Kaplan Comprehensive Cancer Center newsletter, "LAB NOTES". The principal investigator has lectured on the Resource to the Kaplan Comprehensive Cancer Center Core Grant Working Group and at the NYU Breast Cancer Research Program (BCRP). She is also a major participant at the NYU monthly clinical multidisciplinary breast cancer conferences and a member of the Executive Committee of the NYU Breast Center, both of which provide forums for continually updating colleagues on the size of the Resource and the spectrum of available material. Additionally, the Kaplan Comprehensive Cancer Center Breast Cancer Research Program Grant has funded pilot projects for translational research from 1995-1998 generating intramural users (Appendix 3).

Our Internet listing through the National Action Plan has generated 6 outside users of the Resource, one in 1996, three in 1997, and two in 1998.

Based on investigator feedback, our efforts in filling requests for specimens and determining investigator satisfaction with specimens has produced results ranging from good to

excellent. All investigators have been very satisfied with the state in which they have received specimens shipped or delivered to them. Feedback from 1995, 1996, and early 1997 recipients indicates that the material was suitable for the research techniques that they used. An example of such feedback and publications referring to the Resource and its funding source are shown in Appendix 1.

Several slide-based techniques performed in the principal investigator's department and elsewhere in the Medical Center produced good results of immunohistochemistry (Appendix 2) and immunofluorescence microscopy on archived samples. Fluorescent in situ hybridization (Appendix 2) results have been excellent on 1997 samples, and good on 1995 and 1996 samples.

At termination of the grant the Breast Tissue Resource is being transferred to the auspices of the Resource for Tumor Tissue and Data of the Kaplan Comprehensive Cancer Center. Thus, the Resource technician, materials, and database will remain available. Collection of samples will continue and the materials collected will remain available to investigators.

## CONCLUSIONS

The Resource has acquired 2,032 specimens from 1,467 patients.

Requests for snap frozen samples of established breast cancers, matched with normal tissue from the same patient are the most frequent requests received.

Sample preservation is good.

We have met investigator's needs in all instances.

The Resource has provided the principal investigator with outstanding opportunities for ongoing collaboration in various aspects of breast cancer research (1-13).

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#### LIST OF PERSONNEL RECEIVING PAY FROM THIS EFFORT

Helen Feiner, M.D.  
Jaishree Jagirdar, M.D.  
Ms. Yara Delgado

Feiner, Helen, D.  
DAMDM17-94-J-4177

best available copy

APPENDIX 1



**Request for Tissue/Cells**  
**NYU Breast Cancer Resource**  
**Director: Helen D. Feiner, M.D.**  
**560 First Ave. NY, NY 10016**  
**Tel (212) 263-8826**  
**Fax (212) 263-7916**

<b>Name:</b>	<b>Dr. CHARLES CARMECI/ D. THOMPSON Ph.D</b>
<b>Title:</b>	<b>MD/Ph.D</b>
<b>Address:</b>	<b>STANFORD UNIVERSITY. DEPTO OF SURGERY MSLS P229. 1201 WELCH RD STANFORD, CA 94305</b>
<b>Phone:</b>	<b>(415) 725-1671 (415) 498-5510</b>
<b>Fax:</b>	<b>(415) 725-8762</b>
<b>e-mail:</b>	<b>--</b>
<b>Grant Support:</b>	<b>YES</b>
<b>Material Requested:</b>	<b>BREAST TISSUE IN VIAL CONFIRMED ER+ AND ER- Date Shipped: 8-5-96 4-21-97 Date Received: NEXT DAY State of Specimen on receipt: GOOD</b>
<b>Brief Summary of intended use:</b> (Use additional page if necessary)	<b>TO IDENTIFY AND CHARACTERIZE GENES THAT ARE COORDINATELY EXPRESSED WITH ER AND DETERMINE THEIR INFLUENCE ON BREAST CANCER PROTOTYPE.</b>

Charles Carmeci, MD  
Stanford University  
Dept of Surgical Oncology  
MSLS P229  
1201 Welch Rd  
Stanford, CA 94305

8/16/96

CL (415) 725-1671

Helen Feiner, MD  
Dept of Surgical Pathology  
NYU Medical Center  
560 First Avenue  
New York, NY 10016

Dear Dr. Feiner,

Thank you for the primary breast cancer specimens. They arrived in excellent condition.

We have recently isolated and partially characterized several genes from breast cancer cell lines which are coordinately expressed with the gene for estrogen receptor. We feel that this set of genes plays a critical role in determining the differing phenotypes between ER positive and ER negative carcinomas. Using Northern blots from the samples which you have provided, we aim to determine the expression of these genes in primary tumors. The NIH has provided funding for this project (Grant #: NIH/NRSA#1F32CA69715-01A1 PI: Ronald Weigel, MD, PhD).

Thank you for providing such a valuable resource.

*Charles Carmeci*  
Charles Carmeci, MD

*By phone from Dr. Carmeci 5/5/97*  
- yield of 20-30 ng total RNA  
- Not great for Northern  
- RT PCR yield good of all  
ER+ samples and most  
ER- samples. Low  
yield from normal breast tissue  
HT

NEW YORK UNIVERSITY MEDICAL CENTER  
Anatomic Pathology, Room 461  
560 First Avenue  
New York, N.Y. 10016

**F A X**

Date: 1/21/97

Number of pages including cover sheet: 1

TO: DR CHARLES CARMECI FROM: DR HELEN FEINER

Phone: \_\_\_\_\_

Phone: (212) 263-5470

Fax: 415 723-8762

Fax: (212) 263-7916

As discussed, information to add to  
your publication(s) :

REMARKS: ☐ Urgent ☐ For your review ☐ Reply ASAP ☐ Comment

Acknowledgment. Breast cancer tissue  
was obtained from the Breast Cancer  
Resource of the Department of Pathology,  
N.Y.U. Medical Center, Dr Helen  
Feiner, Director. The Resource is funded  
by The Department of the Army, Grant  
DAMD 17-94-J-4177



---

560 First Avenue, New York, N.Y. 10016

Cable Address: NYUMEDIC

---

Department of Pathology

---

(212) 263-

8/12/96

Dr. Charles Carmeci  
Dept of Surgery  
Stanford University  
1201 Welch Rd  
Stanford, CA 94305

Dear Dr. Caremeci:

This is to confirm that on August 5, 1996 we shipped you 18 frozen breast tissue specimens, as follows:

- 8 estrogen receptor positive carcinomas
- 8 estrogen receptor negative carcinomas
- 2 non tumor breast tissue

Please let us know the state in which the specimens were received, a brief statement of the intended use, and how well the material served your purposes.

Many thanks in advance for this important feedback.

Yours sincerely,

A handwritten signature in cursive script, appearing to read "Helen Feiner".

Helen Feiner, M.D.  
Director, Anatomic Pathology  
Director, Breast Cancer Resource  
PH (212) 263-8826 FAX (212) 263-7916

cc: Rita Demopoulos, M.D.

**STANFORD UNIVERSITY SCHOOL OF MEDICINE**

DEPARTMENT OF SURGERY  
MEDICAL SCHOOL OFFICE BLDG. (MSOB),  
SUITE X300  
STANFORD, CALIFORNIA

Ph: ~~415/725-7280~~ FAX No: ~~415/725-3918~~

725-8762

TO: Helen Feiner

PH. NO: (212)263 - 5470

FAX NO: (212)263 - 7916

NO. OF PGS: 3 (Including this page)

FROM: Devon Thompson

PH. NO: (415)498 - 5510

**COMMENTS:**

If you have an email address I  
could forward you the list of tumours  
so you would have it on your computer

*STANFORD UNIVERSITY*  
*Department of Surgery*

1201 Welch Road  
MSLS Building, Room P228  
Stanford, CA 94305-5486  
phone: (415) 498-5510 or (415) 725-1671  
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*Devon A. Thompson, Ph.D.*  
*Postoral Doctoral Fellow*  
*devont@leland.stanford.edu*

Helen Feiner, M.D.  
Department of Surgical Pathology  
N.Y.U. Medical Center  
560 First Avenue,  
New York, NY 10016

July 9th 1997

Dear Dr. Feiner,

I have been working in collaboration with Charles Carmeci, M.D., with whom you have had previous discussions. We have received 31 breast tumour and 4 normal breast samples from your Breast Cancer Tissue Bank. This source has been invaluable to us. We have used these samples to extract RNA and then perform RT-PCR to detect several different genes. At this juncture it would be extremely useful if we could obtain any information you have in your files pertaining to the specific tumours that you have provided to us. Information such as, histological grade, the method(s) used to establish the estrogen receptor phenotype and quantitative values for the ER levels determined. Following I have listed our ID number and your ID number for each of the tumours that we have received.

I will be away on vacation from July 12th until July 26th. You can contact me by e-mail [devont@leland.stanford.edu](mailto:devont@leland.stanford.edu), by phone (415) 498-5510, or fax (415) 725-8762 after this date. Thank you for your help with this matter.

Sincerely,



Devon A. Thompson, Ph.D.  
[devont@leland.stanford.edu](mailto:devont@leland.stanford.edu)

Stanford ID	NYU Tumour ID	Current Information
1	s95 10787	ER + tumour
2	s95 11102	ER + tumour
3	s95 11319	ER + tumour
4	s95 12182	ER + tumour
5	s95 17621	ER + tumour
6	s96 2526	ER + tumour
7	s95 8934	ER + tumour
8	s95 12034	normal breast
9	s95 14255	normal breast
10	s95 14788	ER - tumour
11	s95 21162	ER - tumour
12	s96 1236	ER - tumour
13	s96 2129	ER - tumour
14	s96 2423	ER - tumour
15	s95 10239	ER - tumour
16	s95 12254	ER + tumour
17	s96 12828	ER - tumour
19	s97 4250	ER - tumour
20	s97 4288	ER + tumour
21	s96 20823	ER + tumour
22	s97 2396	ER + tumour
23	s97 4379	ER + tumour
24	s97 3778	ER + tumour
25	s96 20371	ER + tumour
26	s97 4926	ER + tumour
27	s97 596	ER + tumour
28	s96 19122	ER - tumour
29	s97 528	ER - tumour
30	s96 12363	ER - tumour
31	s96 14116	ER - tumour
32	s96 20358	ER - tumour
33	s96 15075	ER - tumour
34	s97 1647	ER - tumour
35	s97 1792	normal breast
		normal breast

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cc:Mail for: Helen Feiner

---

**Subject:** NYU breast cancer samples.

**From:** Helen Feiner 7/24/97 12:50 PM

**To:** devont@leland.stanford.edu at PMDF

---

Dear Dr Thompson,

I have sent you, by mail, two reports from each of the patients listed in your communication of July 9th. One is the surgical pathology report from which you can derive a histologic grade. The most common grading system utilizes architectural grade + nuclear grade + mitotic rate. The second report is from our molecular pathology lab from which you can obtain the estrogen receptor quantitative values. Let me know if you need help with any of these data.

Method used to obtain ER phenotype: Indirect immunoperoxidase technique. Estrogen receptor antibody is obtained from AMAC (clone ER1D5, Westbrook, ME) and Novo Castra (clone 6F11, distributed by Vector, Burlingame, CA). Secondary antibody is horse anti mouse IgG. A standard avidin-biotin-peroxidase technique is used on formalin fixed, paraffin embedded tissue sections. Antibody expression is evaluated in 10 40x fields in a CAS Image Analyzer. Result is expressed as percent positive nuclear area.

Helen Feiner M.D.



# STANFORD UNIVERSITY SCHOOL OF MEDICINE

STANFORD, CALIFORNIA 94305-5486

Devon A. Thompson, Ph.D.  
Postdoctoral Fellow  
Department of Surgery  
Division of Surgical Oncology

MSLS Building, Room P228  
1201 Welch Road  
(650) 498-5510  
(650) 723-8762 (fax)  
email: devont@leland.stanford.edu

Helen Feiner, M.D.  
Department of Surgical Pathology  
N.Y.U. Medical Center  
560 First Avenue,  
New York, NY 10016

August 25 1998

Dear Dr. Feiner,

Thank you for the information pertaining to the breakdown of race status, with regard to patients from whom the breast tumour specimens are obtained. Enclosed are re-prints from some papers in which we have used the tumour specimens that you provided. These frozen tumours have been invaluable to us in extrapolating our findings in breast cancer cell lines to breast tumour biology. We hope to continue using the Breast Cancer Resource of the Department of Pathology, New York University Medical Center, to obtain breast cancer samples.

Sincerely,

A handwritten signature in cursive script, appearing to read "Devon A. Thompson".

Devon A. Thompson, Ph.D.

# PUBLICATIONS THAT ACKNOWLEDGE THE RESOURCE :

Eur. J. Biochem. 252, 169–177 (1998)  
© FEBS 1998

## **Characterization of a gene that is inversely correlated with estrogen receptor expression (ICERE-1) in breast carcinomas**

Devon A. THOMPSON and Ronald J. WEIGEL  
Department of Surgery, Stanford University, Stanford CA, USA

(Received 22 September/10 December 1997) – EJB 97 1350/1

1116–1123 *Nucleic Acids Research*, 1998, Vol. 26, No. 4

© 1998 Oxford University Press

## **Differential screening and suppression subtractive hybridization identified genes differentially expressed in an estrogen receptor-positive breast carcinoma cell line**

Wayne W. Kuang, Devon A. Thompson, Renee V. Hoch and Ronald J. Weigel\*

Department of Surgery, Stanford University, Stanford, CA 94305, USA

Received June 10, 1997; Revised and Accepted December 18, 1997

DDBJ/EMBL/GenBank accession no. AF007170

GENOMICS 45, 607–617 (1997)  
ARTICLE NO. GE974972

## **Identification of a Gene (GPR30) with Homology to the G-Protein-Coupled Receptor Superfamily Associated with Estrogen Receptor Expression in Breast Cancer**

Charles Carmeci,\* Devon A. Thompson,\* Huijun Z. Ring,†  
Uta Francke,†† and Ronald J. Weigel\*,<sup>1</sup>

\*Department of Surgery, †Department of Genetics, and ††Howard Hughes Medical Institute,  
Stanford University, Stanford, California 94305

Received April 4, 1997; Accepted August 11, 1997



**Request for Tissue/Cells**  
**NYU Breast Cancer Resource**  
**Director: Helen D. Feiner, M.D.**  
**560 First Ave. NY, NY 10016**  
**Tel (212) 263-8826**  
**Fax (212) 263-7916**

**Name:** Dr. KEN TAKASHITA

**Title:** ASSISTANT PROFESSOR

**Address:** NYU MEDICAL CENTER  
DEPT. OF HEMATOLOGY

**Phone:** (212) 263-5465

**Fax:** (212) 263-8444

**e-mail:** --

**Grant Support:** YES

**Material Requested:** FROZEN SECTIONS OF  
METASTATIC BREAST CANCER.

**Date Shipped:** 8-15-97

**Date Received:** SAME DAY

**State of Specimen on receipt:** GOOD

**Brief Summary of intended use:**

(Use additional page if necessary)

TO PERFORM IN SITU HYBRIDIZATION AND  
IMMUNOHISTOCHEMISTRY, IN ORDER TO DETERMINE  
WHETHER THE DECREASED EXPRESSION OF RAR-ALPHA  
RETINOIC ACID RECEPTOR EXPRESSION SEEN IN  
METASTATIC BREAST CA. IS DUE TO A TRANSCRIPTIONAL  
DEFECT OR A TRANSLATIONAL DEFECT.

NYU  
Medical  
Center

Hematology Division, Department of Medicine  
New York University Medical Center  
550 First Avenue, New York, N.Y. 10016 U.S.A.

e-mail takeshtk@is.nyu.edu  
Tel 1-212-263-5465, Fax 1-212-263-8444

12/9/97  
Re Dr Takashita & confirmed  
by H.F. on slide review  
Good signed in IHC  
r ISH

August 15, 1997

Dr. Helen Feiner  
Department of Pathology  
Breast Cancer Archives

Dear Dr. Feiner:

I am writing to notify you that we have requested and received from Yara Delgado of the breast tumor registry frozen sections of lymph nodes containing known breast cancer metastasis from 7 different patients. We received 6 slides for each patient.

These sections will be used to perform in situ hybridization and immunohistochemistry. The objective of this experiment is to determine whether the decreased expression of RAR-alpha retinoic acid receptor expression seen in metastatic breast cancer is due to a transcriptional defect or a translational defect.

We are grateful for your assistance in our studies. Please contact me if you have any questions.

Sincerely yours,

*Ken Takeshita*

Ken Takeshita, M.D.  
Assistant Professor of Medicine

Feiner, Helen, D.  
DAMDM17-94-J-4177

best available copy

APPENDIX 2



**NYU BREAST CANCER RESOURCE FOR  
RESEARCH AND BANKING**

Phone: (212) 263 8826-8079

Fax #: (212) 263 7916

**RECORD OF EVALUATION OF BANKED MATERIAL:**

Type of Specimen:      Imprint ✓      Frozen Tissue       

Date of evaluation:

9/4/97

Duration in freezer:

2 years

Type of evaluation:

IMMUNO HISTOCHEMISTRY

Results:

Excellent

Entered by:

HELEN FEINER MD

Signature and date:

*H. Feiner*

9/13/97.

IHC#

## IMMUNOHISTOCHEMISTRY

\* Place completed formin Tisch-379 (ext 8922)RESIDENT/ ATTENDING Dr. FennelPATIENT Anonymous SURG PATH#: 98-45-16437 Block     \*DATE 5/12/98 SITE Breast SPECIMEN: biopsy/ major ImpmtDIAGNOSTIC ISSUE None / QC material

ANTIBODIES : CIRCLE (if limited tissue, number antibodies according to priority, and request "numbered PLL" slides under special requests)

LEU- M1	Calretinin	CK19	GFAP	B72.3	Adenovirus
Muscle Specific	<u>CAM 5.2 (CK)</u>	NSE	*HCG	PSA	*HBsAg
ACTIN				PAP	*HBcAg
DESMIN	AE1/AE3	CHRO		FVIII	CMV
SMA	EMA	*SYN	*AFP	CD34	*HSV
VIMENTIN	34BE12	*CALCITON	pCEA	CD 68	ER/PR
*S-100	CK7	THYRO	mCEA		
HMB45	CK20	*MYOGLOBIN	<u>LCA</u>	BerEP4	Brst-2

# PLL SLIDES REQUESTED        (circle # if ordered on gross sheet)SPECIAL REQUESTS:(CIRCLE): RUSH / USE H&E SECTION /       RCVD        STAINED        SIGNED OUT        TURNAROUND       SPECIAL PROCESSING       IHC INTERPRETATION: NEGATIVE CONTROL- ⊖ POSITIVE CONTROLS - ⊕CAM 5.2 - 3+LCA - 2-3+remedial step(s):        result-        Conclusion-       

ABBREVIATIONS AND CLONE # \* = POLYCLONAL ANTIBODY

SMA= SMOOTH MUSCLE ACTIN (1A4), MUSCLE SPECIFIC ACTIN (HHF-35), AE1/AE3 & CAM 5.2= LOW MOL WT KERATINS, 34BE12= HIGH MOL WT KERATIN, CALC= CALCITONIN, THYRO= THYROGLOBULIN (Tg6), PSAP= PR ACID PHOS (PASE/4LT), PSA= PROSTATE SPECIFIC ANTIGEN (EA-PR8), FVIII=FACTOR VIII (F8/86), SYN= SYNAP TOXIMIN (SYN-1), CEA= (A5B7), EMA= (E29), AFP= ALPHAFETOPROTEIN (M1A1301), HSV= HERPES. PLAP= PLACENTAL ALKALINE PHOSPHATASE (8B6)

HCG= HUMAN CHORIONIC GONADOTROPHIN, HBsAg=HEPATITIS B SURFACE Ag, HBcAg=HEPATITIS B CORE Ag, CD34=(QBEND10.), CD68=(KPI), CK7= OV-TL 12/30, CK20= 1T-KS20.8

Histology:        Date/time submitted        date/time cut



## NYU BREAST CANCER RESOURCE FOR RESEARCH AND BANKING

**Phone: (212) 263 8826-8079**

**Fax #: (212) 263 7916**

**RECORD OF EVALUATION OF BANKED MATERIAL:**

**Type of Specimen:**

**Aspirated Cells \_\_\_\_\_**  
**Imprint \_\_\_\_\_**

## Frozen Tissue ✓

**Date of evaluation:**

September/ 97

**Duration in freezer:**

## 2 YEARS

**Type of evaluation:**

**IMMUNOHISTOCHEMISTRY: MIB-1**  
**p21**

**Results:**

## SATISFACTORY TO GOOD

**Entered by:**

**Dr. H. FEINER**

**Signature and date:**

MF 9/12/97

IHC#

## IMMUNOHISTOCHEMISTRY

\* Place completed formin Tisch-379 (ext 8922)RESIDENT/ ATTENDING H Fine

PATIENT \_\_\_\_\_

SURG PATH#: 98-Block   DATE 9/5/97SITE BreastSPECIMEN: biopsy/ major

DIAGNOSTIC

ISSUE Q A material - (FS)

**ANTIBODIES** : CIRCLE (if limited tissue, number antibodies according to priority, and request "numbered PLL" slides under special requests)

LEU- M1	Calretinin	CK19	GFAP	B72.3	Adenovirus
Muscle Specific	CAM 5.2 (CK)	NSE	*HCG	PSA	*HBsAg
ACTIN	AE1/AE3	CHRO	PLAP	PAP	*HBcAg
DESMIN	EMA	*SYN	*AFP	FVIII	CMV
SMA	34BE12	*CALCITON	pCEA	CD34	*HSV
VIMENTIN	CK7	THYRO	mCEA	CD 68	ER/PR
*S-100	CK20	*MYOGLOBIN	LCA	BerEP4	Brst-2
HMB45					

# PLL SLIDES REQUESTED \_\_\_\_\_ (circle # if ordered on gross sheet)

SPECIAL REQUESTS:(CIRCLE): RUSH / USE H&amp;E SECTION / \_\_\_\_\_

RCVD \_\_\_\_\_ STAINED \_\_\_\_\_ SIGNED OUT \_\_\_\_\_ TURNAROUND \_\_\_\_\_

SPECIAL PROCESSING \_\_\_\_\_

IHC INTERPRETATION: NEGATIVE CONTROL-    POSITIVE CONTROLS- (+)

CK 7 - 2+

remedial step(s):

result-

Conclusion- H Fine

## ABBREVIATIONS AND CLONE #

\* = POLYCLONAL ANTIBODY

SMA= SMOOTH MUSCLE ACTIN (1A4), MUSCLE SPECIFIC ACTIN (HHF-35), AE1/AE3 & CAM 5.2= LOW MOL WT KERATINS, 34BE12= HIGH MOL WT KERATIN, CALC= CALCITONIN, THYRO= THYROGLOBULIN (Tg6), PSAP= PR ACID PHOS (PASE/4LT), PSA= PROSTATE SPECIFIC ANTIGEN (EA-PR8), FVIII=FACTOR VIII (F8/86), SYN= SYNAP CYTOMEGALOVIRUS (DDG9, CCH2), DESMIN (D33), VIMENTIN (V9), LCA= LYMPHOCYTE COMMON ANTIGEN (2B11), CEA= (A5B7), EMA= (E29), AFP= ALPHAFETOPROTEIN (M1A1301), HSV= HERPES, PLAP= PLACENTAL AL PHOSPHATASE (8B6)  
 HCG= HUMAN CHORIONIC GONADOTROPHIN, HBsAg =HEPATITIS B SURFACE Ag, HBcAg=HEPATITIS B CORE Ag CD34=(QBEND10.), CD68=(KPI), CK7= OV-TL 12/30, CK20= IT-K520.8

Histology:

Date/time submitted

date/time cut



**NYU BREAST CANCER RESOURCE FOR  
RESEARCH AND BANKING**

Phone: (212) 263 8826-8079

Fax #: (212) 263 7916

**RECORD OF EVALUATION OF BANKED MATERIAL:**

Type of Specimen:

Imprint ✓

Frozen Tissue \_\_\_\_\_

Date of evaluation:

9/9/98

Duration in freezer:

2 years

Type of evaluation:

FISH

Results:

Attached

Entered by:

J. FEINER

Signature and date:

*[Signature]*

9/18/98



# NEW YORK UNIVERSITY MEDICAL CENTER CYTOGENETICS LABORATORY

New Bellevue Hospital  
Dept. of Pathology/Cytogenetics Lab  
Room 4 North 20  
27th Street & First Avenue, New York, NY 10016

(212) 263-6454  
(212) 562-3496  
Fax: (212) 263-7930

PATIENT S96-20371 CASE # Research-S9620371  
REFERRAL Dr. H. Feiner DATE COMPLETED 9/09/98  
HOSPITAL NYU-Research DATE COLLECTED unknown  
DATE RECEIVED 9/03/98

## MOLECULAR CYTOGENETIC ANALYSIS

SPECIMEN TYPE Tissue Imprints CHROMATID BREAKS \_\_\_\_\_  
QUALITY OF PREPARATION adequate CHROMOSOME BREAKS \_\_\_\_\_  
NO. OF CELLS EXAMINED 50+ ANEUPLOID CELLS \_\_\_\_\_

## INTERPRETATION:

Slides were received from air-dried material described as imprints/scrapes from breast tissue.

Interphase molecular cytogenetic analysis was performed using fluorescent in situ hybridization (FISH) with investigational DNA probes specific for the centromeric region of the X chromosome (Vysis CEP X-alpha probe set). Random sections of the slide were examined by two independent readers. Adequate signal for analysis was seen over the majority of the hybridization area. Results indicated over 85% of cells contained two signals for the X chromosome consistent with two copies of the X. No evidence was seen of X chromosome aneuploidy.

MOLECULAR CYTOGENETIC DIAGNOSIS: nuc ish Xcen(DXZ1x2)

Mary Ann Perle, Ph.D.  
Director, Cytogenetics  
Laboratory

Note: Since this is an in vitro test, accuracy may be limited by technical or cultural artefacts.

Feiner, Helen, D.  
DAMDM17-94-J-4177

### APPENDIX 3

BREAST CANCER PILOT PROJECTS AWARDED  
1995 - 1998

1995 GRANT YEAR

Pamela Cowin, Ph.D. Assistant Professor Cell Biology	"The Role of Plakoglobin in Breast Cancer" (\$30,000)
Xiao-Hong Sun, Ph.D. Assistant Professor Cell Biology	"The Role of ID Proteins in Breast Cancer" (\$28,450)
Mary Ann Perle, Ph.D. Assistant Professor Pathology	"Chromosomes 7, 18, 20 and X in Mammogram Detected Atypical Ductal Hyperplasia and Ductal Carcinoma in situ" (\$8,950)

1996 GRANT YEAR

Sandra Reynolds, Ph.D. Res. Assistant Professor Dermatology	"Peptide Epitopes Recognized by CD8+ T Cells in Patients with Breast Cancer" (\$10,000)
Herbert Samuels, M.D. Professor Medicine	"Retinoid-Regulated Genes and Breast Cancer" (\$25,000)
Jan Sap, Ph.D. Assistant Professor Pharmacology	"Receptor Protein Tyrosine Phosphatases and Breast Cancer" (\$20,000)
Kenichi Takeshita, M.D. Assistant Professor Medicine	"9-cis Retinoic Acid and Retinoid X Receptor RXR in Breast Cancer" (\$20,000)
Stephen Tomlinson, Ph.D. Assistant Professor Pathology	"The Role of Complement Inhibitors in Tumorigenicity" (\$10,000)
Stanislav Vukmanovic, MD, PhD Assistant Professor Pathology	"Effector Function of Vaccine Induced CD8+ Cells" (\$10,000)

1997 GRANT YEAR

Harry Ostrer, M.D. (P.I.) Professor Pediatrics	"Genetic Susceptibility to Breast Cancer" (\$15,000)
Ruth Oratz, M.D. (Co-P.I.) Assistant Professor Medicine	

W. Fraser Symmans, M.D. (P.I.)  
Assistant Professor  
Pathology  
Matthew Volm, M.D. (Co-P.I.)  
Instructor  
Medicine

"A Response Biomarker for Paclitaxel Chemotherapy  
in Patients with Breast Cancer"  
(\$29,875)

Carolyn Wasserheit, M.D. (P.I.)  
Assistant Professor  
Medicine  
Kenichi Takeshita, M.D. (Co-P.I.)  
Assistant Professor  
Medicine

"Biological Correlates of 9-Cis Retinoic Acid and  
Tamoxifen"  
(\$15,000)

1998 GRANT YEAR

Ruben Abagyan, Ph.D.  
Associate Professor  
Biochemistry

"Toward A New Chemotherapy for Breast Cancer:  
Rational Design of A Retinoid X Receptor-Selective  
Agonist"  
(\$29,968)

Alan Frey, Ph.D.  
Assistant Professor  
Cell Biology

"Translational Arrest of IL-2 mRNA in Human Breast  
Cancer Tumor Infiltrating Lymphocytes"  
(\$30,000)

Giorgio Inghirami, M.D.  
Associate Professor  
Pathology

"Molecular Characterization of BRCA1"  
(\$30,000)

Carole Oddoux, Ph.D.  
Assistant Professor  
Pediatrics

"Heritable Susceptibility to Invasive and Non-Invasive Breast  
Cancer"  
(\$15,000)